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Configuration of polyisoprenoids affects the permeability and thermotropic properties of phospholipid/polyisoprenoid model membranes

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ABSTRACT

The influence of α-*cis*- and α-*trans*-polyprenols on the structure and properties of model membranes was analyzed. The interaction of Ficaprenol-12 (α-*cis*-Prenol-12, α-*Z*-Prenol-12) and Alloprenol-12 (α-*trans*-Prenol-12, α-*E*-Prenol-12) with model membranes was compared using high performance liquid chromatography (HPLC), differential scanning calorimetry (DSC) and fluorescent methods. L-α-Phosphatidylcholine from egg yolk (EYPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) as the main lipid components of unilamellar (SUVs) and multilamellar (MLVs) vesicles were used. The two-step extraction procedure (n-pentane and hexane, respectively) allowed to separately analyze the fractions of polyprenol as non-incorporated ($Prenol_{NonInc}$) and incorporated ($Prenol_{Inc}$) into liposomes. Consequently, distribution coefficients, P', describing the equilibrium of prenol content between phospholipid (EYPC) membrane and the aqueous phase gave different $\log P'$ for α-*cis*- and α-*trans*-Prenol-12, indicating that the configuration of the α-terminal residue significantly alters the hydrophobicity of the polyisoprenoid molecule and consequently the affinity of polyprenols for EYPC membrane. In fluorescence experiments α-*trans*-Pren-12 increased up to 1.7-fold the permeability of EYPC bilayer for glucose while the effect of α-*cis*-Pren-12 was almost negligible. Considerable changes of thermotropic behavior of DPPC membranes in the presence of both prenol isomers were observed. α-*trans*-Pren-12 completely abolished the pretransition while in the case of α-*cis*-Pren-12 it was noticeably reduced. Furthermore, for both prenol isomers, the temperature of the main phase transition (T_m) was shifted by about 1 °C to lower values and the height of the peak was significantly reduced. The DSC analysis profiles also showed a new peak at 38.7 °C, which may suggest the concomitant presence of more that one phase within the membrane.

Results of these experiments and the concomitant occurrence of alloprenols and ficaprenols in plant tissues suggest that *cis*/*trans* isomerization of the α-residue of polyisoprenoid molecule might comprise a putative mechanism responsible for modulation of the permeability of cellular membranes.

1. Introduction

Polyprenols are a well known subgroup of polyisoprenoid alcohols described already in the 1960s (Stone et al., 1967). The highest accumulation of polyprenols has been noted in plant photosynthetic tissues, but they have also been detected in wood, seeds, flowers and in bacterial cells (reviewed in Swiezewska and Danikiewicz, 2005). Polyisoprenoid alcohol chains are built of 5–100 and more isoprenoid units creating polymers that differ in the chain-length and/or geometrical configuration. With respect to the structure, the hydrocarbon chain of polyprenols is built of an ω-terminal isoprenoid residue followed by 2 or 3 internal *trans* residues and a stretch of *cis* residues; typical polyprenols – ficaprenols are finally decorated with an α-*cis*-terminal residue (Fig. 1A). In 2007 our group described a new type of polyisoprenoid alcohols of plant origin – alloprenols (Fig. 1B) (Ciepichal et al., 2007).

Abbreviations: Pren-12, polyprenol composed of 12 isoprene units; PrenInc, incorporated prenol; PrenNonInc, non-incorporated prenol.

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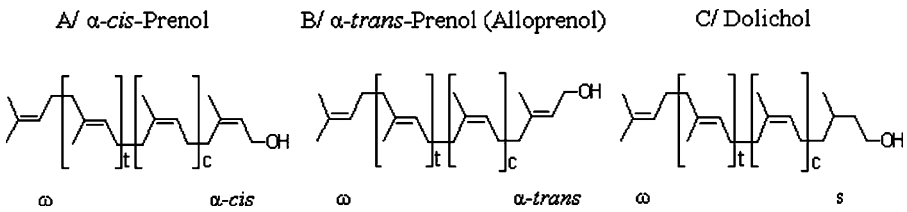

Fig. 1. Structure of A/α-*cis*-polyprenol, B/α-*trans*-polyprenol, C/dolichol; t and c indicate the number of internal *trans*- and *cis*-isoprenoid residues, respectively; ω, α and s stand for ω-, α-terminal and saturated isoprenoid residues, respectively.

In contrast to typical α-*cis* polyprenols (e.g. ficaprenols), alloprenols posses the α-terminal residue in a *trans* configuration. Interestingly, the proportion of the amounts of α-*cis* vs. α-*trans* polyprenols is 1 to 1 in the leaves of *Allophylus caudatus* and eleven other plant species (Marczewski et al., 2007). It should be also mentioned that α-dihydro-polyprenols (syn. dolichols, Fig. 1C) are common constituents of animal and yeast cells.

In contrast to polyisoprenoid phosphates, functioning as cofactors in the biosynthesis of bacterial peptidoglycan and eukaryotic glycoproteins and substrates for protein prenylation (Gutkowska et al., 2004), the role of free polyisoprenoid alcohols is still uncertain. High hydrophobicity of polyisoprenoids causes theirs localization in cellular membranes, e.g. mitochondria, chloroplast envelopes, Golgi membranes (Swiezewska et al., 1993). However, explanation of the presence of polyisoprenoid molecules within the biological membranes has remained for many years a question of debate due to their length which exceeds the thickness of the bilayer. The first molecular model of a dolichol (Murgolo et al., 1989) suggested the dimensions of Dolichol-19 (Dol-19) as 53.07 Å (length) × 30.94 Å (width), and the molecule was proposed to consist of three geometrical regions, a central coiled segment and two flanking regions. According to the recent model the dimensions of Dol-19 were decreased to 31.87 Å (length) × 15.41 Å (width). These findings also revealed that the 3D conformations of Dol-19, Dol-19P (Dolichyl-19-Monophosphate), and Pren-11P (Prenyl-11-Monophosphate) were nearly identical with their three coiled, helical domains (Zhou and Troy, 2003) arranged as a central segment and two flanking arms.

Such a model explains well the possible orientation of the mainly-*cis* polyisoprenoid alcohols in model membranes (Zhou and Troy, 2005). It is also in line with the earlier observations showing that polyprenols, dolichols and their phosphorylated derivatives alter the structure of the phospholipid bilayer by promoting the formation of a nonlamellar (inverted hexagonal, Hex II) structure. These structural changes explained well the effect of polyisoprenoids on the increase of the fluidity, permeability and fusiogenicity of the membranes observed earlier (Chojnacki and Dallner, 1988 and references therein). It has been reported (voltammetric methods) that incorporation of polyprenols into a model lipid membrane (dioleoylphosphatidylcholine, DOPC) decreased the activation energy and increased membrane conductance and membrane permeability coefficient (Janas et al., 2000). However, in all the studies only typical polyisoprenoids with di-*trans*-poly-*cis* or tri-*trans*-poly-*cis* structure were taken into consideration and the effect of discrete structural changes (configuration of the α-*trans*-residue within the prenol molecule) has not been studied yet. This question seems intriguing in the light of the undisclosed biological function of alloprenols.

In this work the influence of α-*cis*-Prenol-12 and α-*trans*-Prenol-12 (Alloprenol-12), occurring concomitantly in plant cell membranes, on the properties of the phospholipid bilayer was analyzed. As model membranes unilamellar (SUVs) and multilamellar (MLVs) vesicles from natural (L-α-phosphatidylcholine, EYPC) or synthetic (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, DPPC) lecithin, were prepared. Efficiency of incorporation of α-*cis*- and *trans*-prenols into unilamellar phospholipid vesicles was examined using quantitative HPLC-UV analysis following the extraction procedure. The effect of polyisoprenoid alcohols on the permeability of model lipid bilayers for glucose, as an example of a low-molecular-weight electrically neutral metabolite, and for protons was monitored by using of fluorescence probes while the influence of polyprenols on thermotropic properties of phospholipid bilayers was followed using differential scanning calorimetry (DSC). Our results indicate that configuration of the polyisoprenoid chain affects the behavior of polyprenols in the bilayer. The occurrence of the additional α-*trans* double bond results in a decreased degree of incorporation of polyprenols into the membrane and at the certain membrane concentration it also results in its enhanced permeability. Interestingly, such a configurational change seems not to be crucial for the formation of lipid domains within the membrane.

2. Materials and methods

2.1. Chemicals

L-α-Phosphatidylcholine from egg yolk (EYPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Prenol-12 – natural α-*cis*-isomer isolated from the leaves of *Magnolia kobus* was from the Collection of Polyprenols (Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland). All solvents of spectral purity and chromatographic materials were from Merck (Darmstadt, Germany). 6-Carboxyfluorescein and valinomycin were from Sigma–Aldrich and pyranine was from Molecular Probes (Eugene, USA). All other chemicals were purchased from Sigma–Aldrich and were of p.a. grade.

2.2. Synthesis of α-trans-Pren-12 (Alloprenol-12)

Aldehyde of Prenol-12 was prepared as earlier (Ciepichal et al., 2007) with some modifications. Briefly: 60 mg of Polyprenol-12 in 1 ml of dichloromethane was stirred with 150 mg of pyridinium chlorochromate for 20 min at room temperature. Aldehyde was isolated by column chromatography (Florisil, dichloromethane). Fractions containing aldehyde (55 mg, mixture of α-*cis*/*trans* aldehydes, 9:1 ratio) were pooled, evaporated under nitrogen (30 °C) and dissolved in 3 ml of methanol. Enhanced isomerization of the α-unit of aldehyde was achieved by the incubation of Prenal-12 in the presence of sodium carbonate (molar ratio of aldehyde:sodium carbonate, 1:15) at room temperature for 16 h (60% conversion). Further reduction of Prenal-12 (mixture of α-*cis*/*trans* aldehydes) with sodium borohydride (molar ratio of aldehyde:sodium borohydride, 2:1) in the same solution (40 min, room temperature) yielded a mixture of α-*cis*- and α-*trans*-Pren-12 (99% conversion). Mixture of α-*cis*- and α-*trans*-Pren-12 was extracted (hexane:water; 1:1), concentrated and products were separated by column chromatography (silica gel 60 column, linear gradient of diethyl ether in hexane from 0 to 10%). Each step of preparation of α-*trans*-Pren-12 was followed by TLC (silica gel plates, ethyl acetate:toluene, 1:9

Table 1
Distribution of α-*cis*-Pren-12 and α-*trans*-Pren-12 between unilamellar EYPC liposomes and aqueous phase. The distribution coefficient $P' = m_{PrenInc}/m_{PrenNonInc}$. Pren-12 content in the initial lipid mixture was within the range from 10 to 100 mol%, which corresponds to polyprenol:EYPC molar ratio in the range from 1:10 to 1:1. The quantitative evaluation of the amount of prenols, $Pren_{Inc}$ and $Pren_{NonInc}$, was assayed by means of HPLC analysis on the basis of integrated peak areas from HPLC elution profiles. Data are means of three independent experiments ± SD. Abbreviation: $Pren_{Inc}$, incorporated prenol; $Pren_{NonInc}$, non-incorporated prenol.

Pren-12 content mol%	10	20	30	40	50	100
			log P'			
α-*cis*-Pren-12	0.16 ± 0.02	0.31 ± 0.03	0.19 ± 0.02	−0.01 ± 0.03	0.00 ± 0.02	0.20 ± 0.09
α-*trans*-Pren-12	−0.66 ± 0.03	−0.79 ± 0.01	−0.68 ± 0.03	−0.61 ± 0.08	−0.72 ± 0.04	−0.75 ± 0.02

(v/v), stained with iodine vapors). The purity of α-*trans*-Pren-12 was finally confirmed using HPLC/Photodiode Array detector with a dual pump apparatus (Waters Ass., USA) using a Hypersil ODS reversed-phase column (Agilent, USA) (4.6 mm × 60 mm, particle size 3 μm) as described earlier (Skorupinska-Tudek et al., 2003). The structure of thus obtained α-*trans*-Pren-12 was confirmed by HPLC/ESI-MS (Pren-12 with a molecular formula $C_{60}H_{98}O$ gave a pseudomolecular ion peak *m*/*z* 857.7 ($[M+Na]^+$)) and by means of ^1H and ^{13}C NMR as previously described (Ciepichal et al., 2007).

2.3. HPLC analysis of the capacity of liposomes for α-cis- and α-trans-Pren-12

Small unilamellar vesicles (SUVs) were prepared at room temperature by the injection method (Kruk et al., 1997). The evaporated lipid mixture of egg yolk phosphatidylcholine supplemented with either α-*cis*- or α-*trans*-Pren-12 was kept under vacuum for 2–3 h, dissolved in ethanol and injected under continuous stirring into 10 mM Hepes buffer (pH 7.7) containing 50 mM KCl and 1 mM EDTA. The final EYPC concentration was 0.5 mM, ethanol concentration of 1.25% and polyprenol content in the lipid mixture in the range of 2–100 mol%, which corresponds to the polyprenol:EYPC molar ratio in the range of 1:50–1:1.

Subsequently, a two-step extraction procedure was performed. Non-incorporated polyisoprenoids ($Prenol_{NonInc}$) were removed with a brief n-pentane washing as described earlier (Degli Esposti et al., 1981). The aqueous phase was then subjected to further extraction according to the published method (Kröger, 1978) with the subsequent modification (Jemioła-Rzemińska et al., 2001) aimed to solubilize liposomes and extract polyisoprenoid alcohols localized in the membrane ($Prenol_{Inc}$). The resulting organic fractions (n-pentane and hexane) were collected and evaporated separately, the obtained residues were dissolved in chloroform:methanol (2:1, v/v). HPLC analysis was performed as described above. All these experimental steps were performed at 21 °C. Estimated amounts of Pren-12 were used for calculation of the distribution coefficient $P' = m_{PrenInc}/m_{PrenNonInc}$ thus higher negative values of log P' (Table 1) indicate elevated accessibility of Pren-12 to pentane.

Measurements were performed in triplicate, values were calculated as mean ± SD.

2.4. Permeability experiments – parallel assays for protons and glucose

Glucose and proton permeability measurements were performed in parallel using aliquots of the same batch of vesicles according to the method published earlier (Berglund et al., 2004). Previous experiments confirmed that there was no interference between carboxyfluorescein and pyranine when present concurrently inside the vesicles (Berglund et al., 2002). In a test tube, to the mixture of EYPC and either α-*cis*- or α-*trans*-Pren-12 dried under N_2, 0.5 ml of 10 mM HEPES–NaOH buffer (pH 7.7) containing 1 mM EDTA, 50 mM KCl, 1 mM carboxyfluorescein and 2 mM pyranine was added. The lecithin content was 5 mg and the content of α-*cis*- or α-*trans*-Pren-12 was within the range of 1–25 mol%. The sample was vortexed for 5–10 min and the vesicles were subjected to five cycles of the freeze–thaw procedure (Mayer et al., 1985). Obtained multilamellar vesicles were converted to unilamellar vesicles by a high pressure extrusion technique (Olson et al., 1979). Each sample was extruded seven times through a double polycarbonate filter, pore size 200 nm, using a Liposofast Basic Equipment (Avestin, Ottawa, Canada). To eliminate proton gradient, valinomycin (1.3 mM) was added and the vesicles were finally incubated overnight at 4 °C (Clement and Gould, 1981). Gel exclusion chromatography on a G-75 Sephadex column (0.5 cm^2 × 15 cm) was performed in order to separate unilamellar vesicles from non-trapped fluorescence dyes. The liposomal preparation was divided into three fractions of 1 ml each and these aliquots were subsequently used for the permeability experiments.

Proton permeability was measured upon addition of 66 μl 0.1 M HCl to the vesicle suspension, with instant mixing. The permeation of protons across the membrane was recorded as a decrease in the fluorescence of pyranine monitored at 508 nm under 460 nm excitation.

Glucose permeability was measured upon addition of 1 ml 1.6 M glucose to 1 ml vesicle suspension. The increase in fluorescence was monitored at 515 nm under 494 nm excitation.

Carboxyfluorescein was used in the glucose permeability experiments as a fluorescence marker due to its self-quenching effect at high concentrations. Upon addition of glucose to the vesicle suspension an immediate shrinkage of the vesicles takes place as a result of water efflux. Rapid increase in the concentration of carboxyfluorescein inside the vesicles is the reason of quenching and decrease in the fluorescence intensity. When glucose gradually permeates through the lipid bilayer, concomitant with water, vesicles regain their size due to the water influx and fluorescence is gradually increased. The recorded constant value of the fluorescence intensity is reached when vesicles recapture their original volume.

Relative permeability of the vesicles for glucose and protons was calculated as described previously (Berglund et al., 2000; Berglund et al., 2004), permeability coefficient for control (pure EYPC vesicles) was set to 1.0.

Fluorescence measurements were performed in a 1 cm quartz cuvette at 25 °C using a Perkin Elmer LS 50B spectrofluorometer.

Measurements were performed in triplicate, values were calculated as mean ± SD.

2.5. Differential scanning calorimetry (DSC) analysis

The multilamellar vesicles (MLVs) were prepared from DPPC and either α-*cis*- or α-*trans*-Pren-12 as described previously (Jemiola-Rzeminska et al., 2002). The final DPPC concentration was 1 mM and α-*cis*- or α-*trans*-Pren-12 content in the samples was in the range of 1–50 mol%.

DSC experiments were performed using a CSC Model 6100 Nano II differential scanning calorimeter (Calorimetry Sciences Corporation, USA). The sample cell was filled with about 400 μl of MLV suspension and an equal volume of distilled water was used as a reference. The data were collected in the range of 25–55 °C at the

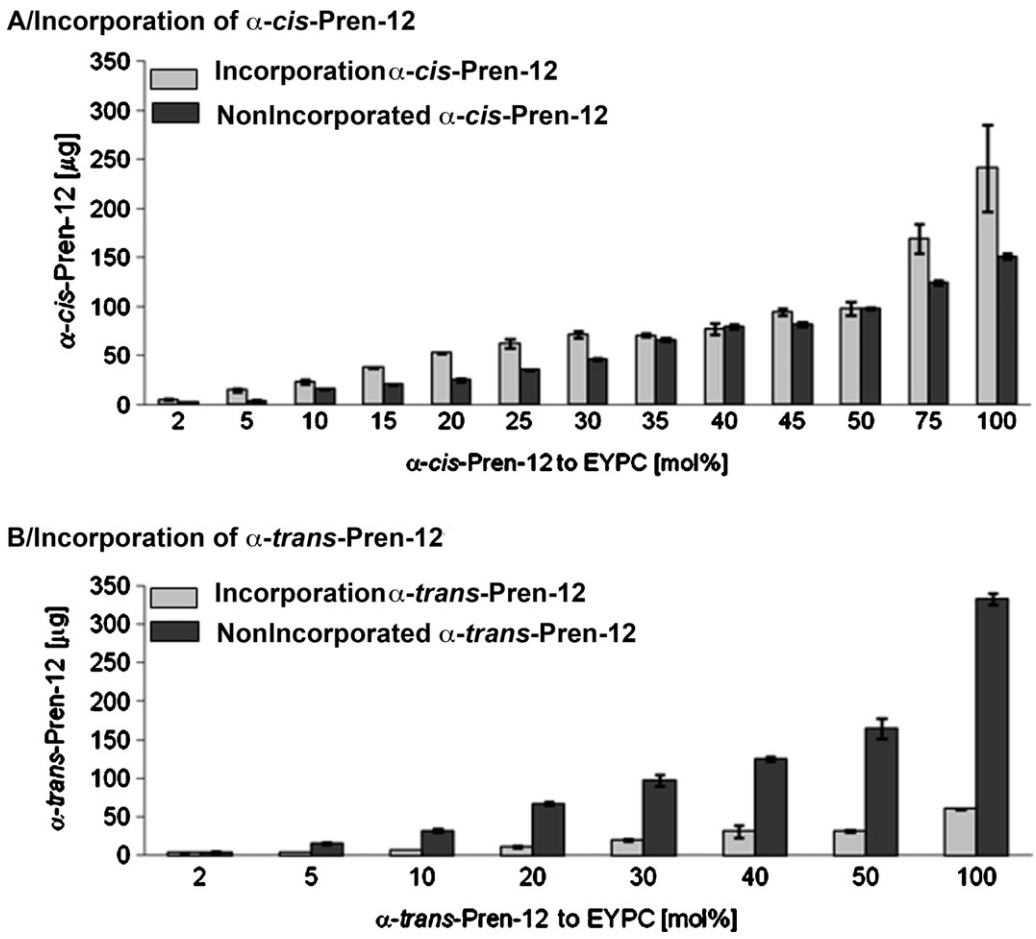

Fig. 2. Incorporation of A/α-*cis*-Pren-12 and B/α-*trans*-Pren-12 into EYPC unilamellar vesicles. The quantitative evaluation of the amount of polyprenols was performed on the basis of the integrated peak areas from HPLC elution profiles. Means of three independent experiments ± SD are shown.

scan rate of 1 °C min^{-1} both for heating and cooling. Measurements were performed in triplicate, representative graphs are presented.

3. Results

3.1. Incorporation of polyprenols into lipid bilayers

Concomitant occurrence of equal amounts of typical α-*cis*-together with newly described α-*trans*-polyprenols in leaves of some plants raised the question of the effect these latter polyisoprenoids wielded on lipid membranes. Because of the limited availability of natural α-*trans*-Prenol-12, a suitable semi-synthetic method was established, as described in Section 2. This approach was based on the fact that alkaline treatment of the molecule containing conjugated double bonds system (–C=C–C=O in the aldehyde, Prenal-12) results in *cis* to *trans* isomerization.

The effect of the configuration of the α-isoprenoid residue on the incorporation efficiency of polyprenols into the unilamellar lecithin vesicles was analyzed. The two-step extraction procedure followed by the HPLC analysis enabled us to compare the amount of polyprenols incorporated into the lipid bilayer with that not accommodated within the membrane interior during vesicle formation. As shown in Fig. 2A, the amount of both, α-*cis*- and α-*trans*-Pren-12 incorporated into the liposomes increased with the increasing Pren-12 to EYPC molar ratio. The incorporation of prenol towards membranes upon such experimental conditions might be discussed as distribution between the phospholipid (EYPC) membrane and the aqueous phase. Distribution coefficients describing the equilibrium of prenol content between phospholipid (EYPC) membrane and the aqueous phase, calculated as $P' = m_{PrenInc}/m_{PrenNonInc}$ (where m represents the amount (g) of prenol recovered from each phase) gave the $\log P'$ mean value -0.70 ± 0.06 for α-*trans*-Prenol-12 (Table 1) indicating that the distribution of α-*trans*-Prenol-12 is independent on the prenol concentration in the initial lipid mixture. In contrast, the $\log P'$ values calculated for incorporation of α-*cis*-Prenol-12 into EYPC vesicles presented a complicated profile with three distinct regions. Namely, for the lowest α-*cis*-Pren-12 concentration (below 30 mol%) almost linear increase of its content in the two phases was observed while for the middle values of the α-*cis*-Pren-12 concentration (above 35 and below 50 mol%) its amounts were almost equal in both phases. Surprisingly, strong increase of incorporation of α-*cis*-Pren-12 into the lipid membrane was observed at its highest concentrations (75 and 100 mol%) (Table 1 and Fig. 2).

3.2. Effect of polyprenols on the permeability of model membranes

Literature data on the effect of dolichols and polyprenols on induction of pore formation resulting in the increased permeability of model membranes prompted us to measure the influence of polyprenols on the permeability of the egg yolk lecithin membrane using the fluorescence-based method (Berglund et al., 2002). Simultaneous incorporation into the liposomal structure of two fluorescent markers: carboxyfluorescein, due to its selfquenching effect at high concentrations (Weinstein et al., 1981), and pyranine, which is a pH dependent fluorescent probe, enabled parallel measurements of vesicle permeability for glucose and protons. Control

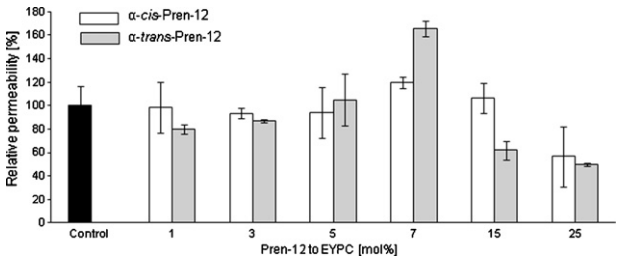

Fig. 3. The influence of α-*cis*-Pren-12 and α–*trans*-Pren-12 on the permeability of model EYPC membranes for glucose. The data represent the means of three independent experiments ± SD.

experiments revealed that incorporation of polyprenols into the egg yolk lecithin bilayer did not change fluorescence parameters such as the excitation or the emission wavelengths neither of carboxyfluorescein not pyranine. What is even more important, no fluorescence quenching was observed in such system (not shown).

In Fig. 3 the effect of Pren-12 in α-*cis*- and α-*trans*- configuration on the permeability of EYPC bilayer for glucose is presented. While α-*cis*-Pren-12 induced almost no changes, the influence of α-*trans*-Pren-12 was strongly dependent on its content. At concentrations lower than 5 mol% it caused strong fluctuations and even the decrease in permeability for glucose, and this effect was even more pronounced for the highest concentrations (15 and 25 mol%). Interestingly, for vesicles containing 7 mol% of α-*trans*-Pren-12 a significant increase in the permeability, reaching 170% of the control value determined for EYPC model membrane was observed.

Pilot measurements of the permeability of EYPC bilayer for protons revealed increased values for liposomes containing higher concentrations (7–25 mol%) of polyprenols (180% of control for liposomes containing 7 mol% of α-*trans*-Pren-12). However, significant conclusions cannot be drawn from these preliminary results because of the relatively broad dispersion of the measured values. Similarly, modest increase of permeability for protons resulted from the incorporation of either all-*trans*-Pren-9 or α-*cis*-mainly-*trans*-Pren-9 into EYPC liposomes (data not shown).

3.3. *The effect of Prenol-12 isomers on the thermotropic behavior of DPPC membranes*

The high-sensitivity DSC heating profiles obtained for DPPC dispersions in excess of water with increasing concentrations of polyprenols are shown in Fig. 4. In the thermal range of 25–50 °C for the pure DPPC multilamellar vesicles we found two thermotropic phase transitions: a highly cooperative gel-to-liquid-crystal transition ($P_{B'} \rightarrow L_\alpha$) at 42.2 °C with an enthalpy change of 8.5 kcal mol^{-1} and the so called pretransition at 34.6 °C with ΔH of 0.9 kcal mol^{-1} ($L_{B'} \rightarrow P_{B'}$), which is consistent with the literature data (Koynova and Caffrey, 1998). The presence of polyprenols significantly altered the thermotropic behavior of DPPC. With the content as low as 1 mol% of α-*cis*-Pren-12, the pretransition was considerably reduced, while in the case of α-*trans*-Pren-12 it was completely abolished and no longer detected for any higher polyprenol concentration.

Moreover, the temperature of the main phase transition (T_m) was shifted by about 1 °C to lower values and the height of the peak was reduced significantly, the effects being more pronounced for the highest (15 and 50 mol%) polyprenol concentrations. Upon analysis of the DSC profiles depicted in Fig. 4, it is also clearly seen that concurrently with a significant broadening of the main phase transition peak and the disturbance of its symmetry, a new peak appeared at 38.7 °C (for Pren-12 content higher than 5 mol%), which may suggest the concomitant presence of more that one phase within the membrane.

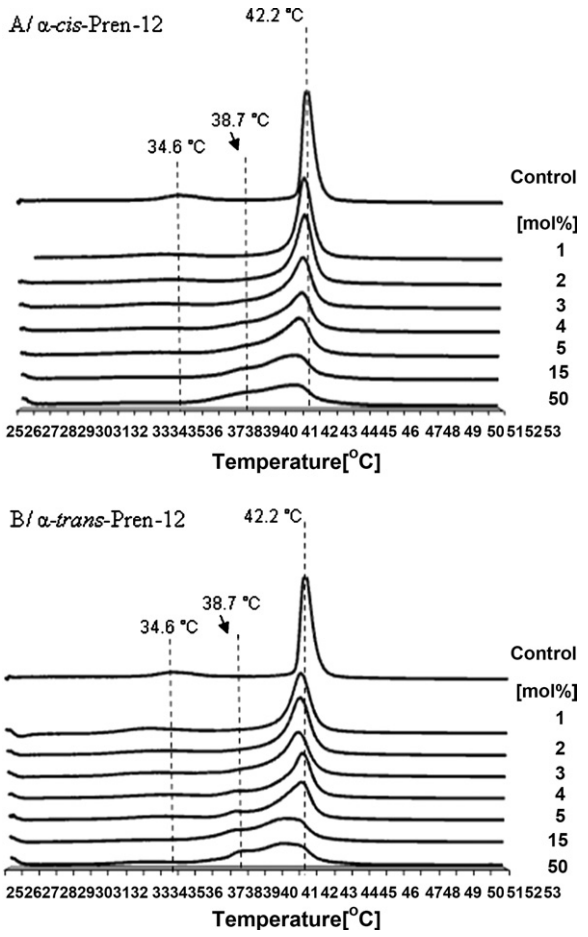

Fig. 4. DSC heating profiles obtained for multilamellar DPPC liposomes containing variable amounts of A/α-*cis*-Pren-12 and B/α-*trans*-Pren-12. The arrow indicates a new signal appearing at 38.7 °C. Scans were obtained at a heating rate of 1 °C min^{-1} and are representatives of the three independent runs.

The thermotropic behavior of the DPPC liposomes containing polyprenols observed during the successive cooling scans (after 10 min of equilibration at 60 °C) in the temperature range from 60 to 20 °C showed the peak characteristic of the $L_\alpha \rightarrow P_{B'}$ transition (data not shown). However, the transition pathways in heating and cooling runs displayed significant hysteresis. The slightly lower excess heat capacity values recorded during cooling may indicate that molecular rearrangements have a substantially longer response time. The pattern observed for cooling of the DPPC/polyprenol systems was essentially similar to the corresponding heating one with a progressive broadening of main transition peak, its shift to the lower temperatures as well as the occurrence of the additional low-temperature peak at high polyprenol concentrations.

4. Discussion

Polyprenols and dolichols are ubiquitous components of all living cells. Their widespread distribution in biomembranes raises the question of their physiological role as modulators of membrane properties. The apparent increase in content of polyisoprenoid alcohols upon tissue senescence and during plant resistance response to biotic stress (Bajda et al., 2009) prompted us to reinvestigate the basis of the interaction between polyisoprenoid alcohols and the lipid matrix. The way in which model membranes are perturbed by introduced polyprenols can be a useful indication of how they interact with the lipid bilayer with a subsequent influence on the membrane properties and relevance for a variety of pro-

cesses which occur in biological systems. Increase of membrane permeability seems crucial for cellular response and adaptation to adverse conditions since environmental stress causes an enhanced exchange of metabolites through cellular compartments. Alteration of membrane fluidity and permeability might be achieved by the adjustment of the content of polyisoprenoid alcohols. In the current report the effect of polyprenols on the permeability and thermotropic properties of membranes was elucidated focusing on the recently discovered new isomeric forms of polyprenols (α-*trans*-polyprenols).

Three types of experiments were performed. At first the efficiency of incorporation of polyprenols into the unilamellar EYPC vesicles was analyzed. There are several lines of evidence proving that polyprenols and dolichols are incorporated into the phospholipid bilayer (Zhou and Troy, 2003, 2005; Valtersson et al., 1985; Wang et al., 2008). However, to the best of our knowledge this is the first quantitative analysis of this phenomenon. The two-step extraction method applied in this study was aimed at differentiation between the pool of Pren-12 molecules incorporated into the membrane and those which were associated with the liposomal surface. Quantification of the Pren-12 fraction easily removed by the mild pentane washing vs. total content of Pren-12 permitted comparison of the incorporation of both isomers of Pren-12 into the EYPC bilayer. Interestingly, different values of the distribution coefficient found for the Pren-12 isomers, $\log P'$, reveal that the configuration of the α-terminal residue significantly alters the hydrophobicity of the polyisoprenoid molecule and consequently the affinity of polyprenols for the EYPC membrane. Observed differences indicate much higher partition of the α-*cis*- than that of α-*trans*-Pren-12 between the phospholipid region and water phase, however the mode of interaction of former isomer with phospholipids seems to be concentration-dependent. Especially strong increase of the α-*cis*-Pren-12 membrane incorporation observed at its high concentrations might suggest structural changes of the vesicles however direct experimental data are required to explain this observation.

In the second set of experiments it was shown that α-*trans*-Pren-12 noticeably modified the permeability of unilamellar egg yolk phosphatidylcholine liposomes for glucose (an increase up to 1.7-fold for 7 mol%) while the effect of α-*cis*-Pren-12 was almost negligible. According to the preliminary results both isomers increased the permeability of liposomes for protons (at 7 mol% content of Pren-12). These data are in agreement with earlier studies based on the electrophysiological technique, which showed that Pren-11 (undecaprenol) increased the permeability coefficients of macrovesicular dioleoylphosphatidilcholine membranes (hemispheric bilayers) for Na^+ and Cl^- ions. It was concluded that undecaprenol substantially decreased the energy barrier for ion migration through membranes resulting in increased ionic conductance (Janas et al., 1994). Similarly, an increase of ion (Cl^-) permeability measured in the presence of transmembrane potential, was observed for Pren-16 incorporated into DOPC bilayers (Janas et al., 2000). However, similar experiments performed for Pren-16 diphosphate revealed the increase of the activation energy of ion migration (up to approx. 1.8-fold) in DOPC bilayer (Janas and Walińska, 2000) and Pren-11P decreased DOPC membrane conductance and ionic permeability (Janas et al., 1994).

Increased permeability of the membrane most probably results from structural perturbations which occur in the membrane due to the incorporation of polyisoprenoid alcohols. This aspect was studied within the third set of experiments where thermotropic behavior of the dipalmitoylphosphatidylcholine multilamellar vesicles supplemented with various amounts of α-*cis*- or α-*trans*-Pren-12 was analyzed using the DSC technique. Incorporation of both Pren-12 isomers resulted in a broadening of the phospholipid main transition, which may reflect a reduction of the cooperativity between the lipid acyl chains. Together with the small but significant decrease of the temperature, this effect is undoubtedly an indication that polyprenols enter the hydrocarbon region of DPPC multibilayers. Strong influence of polyprenols on the pretransition may be explained in terms of greater distortion of the DPPC packing in the gel state. With high α-*trans*-Pren-12 contents, apart from the modifications observed in the shape of the main phase transition peak, an additional peak appeared, which may suggest the existence of spatial non-homogeneity (microdomains), or phase separation. It seems plausible that this observation is related with an intriguing increase of the membrane permeability for glucose and protons observed at 7 mol% content of Pren in the bilayer. Hovewer, more in-depth studies are required to prove it. Such an effect has never been observed for dolichol-doped PC liposomes. A previous report described only a minor decrease of T_m (Vigo et al., 1984) or even no effect of dolichols: Dol-20 (Valtersson et al., 1985) and Dol-19 (Wang et al., 2008) on phospholipid bilayers. However, observations recorded for dolichyl-P showed that it dramatically perturbed DMPC thermotropic behavior leading to the abolition of the transition from the gel to the liquid crystalline phase.

It is also worth mentioning that the literature data reveal a strong relationship between the influence exerted by dolichols on phosphatidylethanolamine (PE) liposomes and the structure of the fatty acyl chains of PE. In the case of DPPE a progressive increase in the temperature of gel to liquid-crystal phase transition was observed upon incorporation of increasing amounts of Dol-19 (Wang et al., 2008) while destabilization of the PE bilayer accompanied by promoted formation of inverted hexagonal phase (H_{II}) was reported for unsaturated PE (Valtersson et al., 1985; Wang et al., 2008).

Dissimilar effects of Pren-12 and dolichols on PC membranes might be explained on the one hand by the difference in their length which affects the amphipatic balance and makes it more or less favorable for hydrophilic affinity. For prenyllipids such as plastoquinones and ubiquinones (Jemiola-Rzeminska et al., 2002; Skowronek et al., 1996; Jemiola-Rzeminska et al., 1996) as well as for dolichol derivatives (Valtersson et al., 1985) the magnitude of bilayer destabilization was shown to depend on the number of isoprene residues. Interestingly, while an increase in the number of isoprene units caused enhanced transversal penetration, at concentrations higher than 1–2 mol%, the gradually increasing fraction of long-chain ubiquinone-10 (UQ-10, coenzyme Q) was found in an aggregated state in the bilayer midplane region. On the other hand, the difference in the geometry of the α-isoprene residue might perhaps also have an impact on the final interaction between polyisoprenoid and fatty acid chains.

It seems reasonable to discuss our DSC and permeability data on the basis of location of the polyisoprenoid molecules within the lipid bilayer which, however, is still being debated. Earlier studies, based on EPR and NMR data, suggested a model in which dolichol (Dol-19) was sandwiched between the two faces of a phospholipid matrix with both ends of the polyisoprenoid molecule localized in the bilayer interior (Valtersson et al., 1985; McCloskey and Troy, 1980; de Ropp and Troy, 1985). A model published recently predicts that polyisoprenoid chains (Dol-19 and Dol-19P) are in close apposition with the acyl chains of the host phospholipids (Zhou and Troy, 2005). The preferred orientation of Dol-19 is primarily parallel to the plane of the bilayer, whereas Dol-19P is preferentially arranged more perpendicular to the plane of the bilayer. Data reported here, i.e. the observed effect of Pren-12 isomers on the pre- and main phase transition of DPPC are consistent with this model and point to the fact that Pren-12 and Dol-19 alike interact with the phospholipids mainly through hydrophobic interactions between the coil region of the polyisoprenoid chain and the fatty acyl chains of the phospholipids.

Consistent results obtained in this study showing the effects induced by intercalation of Pren-12 isomers into PC bilayers, i.e. decreased stability and modulated permeability of these membranes support earlier suppositions that membrane topology may be controlled by polyisoprenoid molecules. Different affinity of α-*cis* vs. *trans*-Pren-12 for phospholipid bilayers might possibly also suggest the possible effect of polyprenols on lateral segregation of lipids in model and biological membranes, as predicted for cholesterol (Slotte, 2009). Consequently, it seems plausible to presume the existence of polyisoprenoid-induced channels in biological membranes. The radius of such pores, induced by Pren-16 in POPC bilayers, estimated from a decrease in the activation energy of ion migration across the lipid bilayers, was predicted to be 2 nm (Janas et al., 2000). Such channels might be of functional importance for intracellular trafficking of intermediates and metabolites, e.g. glycoconjugate translocation during biosynthesis of glycoproteins and peptidoglycans. Furthermore, although different effect of the α-isomers (*trans* vs. *cis*) of Pren-12 on membrane permeability for glucose requires more detailed studies it nevertheless might point towards isomerization of polyprenols as the putative mechanism responsible for regulation of the permeability of cellular membranes.

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References

Bajda, A., Konopka-Postupolska, D., Krzymowska, M., Hennig, J., Skorupinska-Tudek, K., Surmacz, L., Wojcik, J., Matysiak, Z., Chojnacki, T., Skorzynska-Polit, E., Drazkiewicz, M., Patrzylas, P., Tomaszewska, M., Kania, M., Swist, M., Danikiewicz, W., Piotrowska, W., Swiezewska, E., 2009. Role of polyisoprenoids in tobacco resistance against biotic stresses. Physiol. Plant. 135, 351–364.

Berglund, A.H., Norberg, P., Quartacci, M.F., Nilsson, R., Liljenberg, C.S., 2000. Properties of plant plasma membrane lipid models—bilayer permeability and monolayer behaviour of glucosylceramide and phosphatidic acid in phospholipids mixtures. Physiol. Plant. 109, 117–122.

Berglund, A.H., Quartacci, M.F., Calucci, L., Navari-Izzo, F., Pinzino, C., Liljenberg, C., 2002. Alterations of wheat root plasma membrane lipid composition induced by copper stress result in changed physicochemical properties of plasma membrane lipid vesicles. Biochim. Biophys. Acta 1564, 466–472.

Berglund, A.H., Larsson, K.E., Liljenberg, C.S., 2004. Permeability behaviour of lipid vesicles prepared from plant plasma membranes—impact of compositional changes. Biochim. Biophys. Acta 1682, 11–17.

Chojnacki, T., Dallner, G., 1988. The biological role of dolichol. Biochem. J. 251, 1–9.

Ciepichal, E., Wojcik, J., Bienkowski, T., Kania, M., Swist, M., Danikiewicz, W., Marczewski, A., Hertel, J., Matysiak, Z., Swiezewska, E., Chojnacki, T., 2007. Alloprenols: novel α-*trans*-polyprenols of *Allophylus caudatus*. Chem. Phys. Lipids 147, 103–112.

Clement, N.R., Gould, J.M., 1981. Pyranine (8-hydroxy-1,3,6-pyrenetrisulfonate) as a probe of internal aqueous hydrogen ion concentration in phospholipid vesicles. Biochemistry 20, 1534–1538.

Degli Esposti, M., Bertoli, E., Parenti-Castelli, G., Fato, R., Mascarello, Lenaz, G., 1981. Incorporation of ubiquinone homologs into lipid vesicles and mitochondrial membranes. Arch. Biochem. Biophys. 210, 21–32.

de Ropp, J.S., Troy, F.A., 1985. ^{2}H NMR investigation of the organization and dynamics of polyisoprenols in membranes. J. Biol. Chem. 260, 15669–15674.

Gutkowska, M., Bieńkowski, T., Vo Si Hung, Wanke, M., Hertel, J., Danikiewicz, W., Swiezewska, E., 2004. Proteins are polyisoprenylated in *A. thaliana*. Biochem. Biophys. Res. Commun. 322, 998–1004.

Janas, T., Chojnacki, T., Swiezewska, E., Janas, T., 1994. The effect of undecaprenol on bilayer lipid membranes. Acta Biochim. Pol. 41, 351–358.

Janas, T., Nowotarski, K., Gruszecki, W.I., Janas, T., 2000. The effect of hexadecaprenol on molecular organization and transport properties of model membranes. Acta Biochim. Pol. 47, 661–673.

Janas, T., Walińska, K., 2000. The effect of hexadecaprenyl diphosphate on phospholipid membranes. Biochim. Biophys. Acta 1464, 273–283.

Jemiola-Rzeminska, M., Kruk, J., Skowronek, M., Strzalka, K., 1996. Location of ubiquinone homologues in liposome membranes studied by fluorescence anisotropy of diphenyl-hexatriene and trimethylammonium-diphenyl-hexatriene. Chem. Phys. Lipids 79, 55–63.

Jemioła-Rzemińska, M., Latowski, D., Strzałka, K., 2001. Incorporation of plastoquinone and ubiquinone into liposomes membranes studied by HPLC analysis. The effect of side chain length and redox state of quinone. Chem. Phys. Lipids 110, 85–94.

Jemiola-Rzeminska, M., Myśliwa-Kurdziel, B., Strzałka, K., 2002. The influence of structure and redox state of prenylquinones on thermotropic phase behavior of phospholipid in model membranes. Chem. Phys. Lipids 114, 169–180.

Koynova, R., Caffrey, M., 1998. Phases and phase transitions of the phosphatidylcholines. Biochim. Biophys. Acta 1376, 91–145.

Kruk, J., Jemiola-Rzeminska, M., Strzalka, K., 1997. Plastoquinol and α-tocopherol quinol are more active than ubiquinol and α-tocopherol in inhibition of lipid peroxidation. Chem. Phys. Lipids 87, 73–80.

Kröger, A., 1978. Determination of contents and redox state of ubiquinone and menaquinone. In: Fleischer, S., Packer, L. (Eds.), Methods in Enzymology, vol. 53. Academic Press, New York, pp. 580–591.

Marczewski, A., Ciepichal, E., Le Xuan Canh, Tran The Bach, Swiezewska, E., Chojnacki, T., 2007. The search for polyprenols in dendroflora of Vietnam. Acta Biochim. Pol. 54, 727–732.

Mayer, L.D., Hope, M.J., Cullis, P.R., 1985. Solute distribution and trapping efficiences observed in freeze-thawed multilamellar vesicles. Biochim. Biophys. Acta 817, 193–196.

McCloskey, M.A., Troy, F.A., 1980. Paramagnetic isoprenoid carrier lipids. 2. Dispersion and dynamics in lipid membranes. Biochemistry 19, 2061–2066.

Murgolo, N.J., Patel, A., Stivala, S.S., Wong, T.K., 1989. The conformation of dolichol. Biochemistry 28, 253–260.

Olson, F., Hunt, C.A., Szoka, F.C., Vail, W.J., Papahadjopoulos, D., 1979. Preparation of liposomes of defined size distribution by extrusion through polycarbonate membranes. Biochim. Biophys. Acta 557, 9–23.

Skorupinska-Tudek, K., Bienkowski, T., Olszowska, O., Furmanowa, M., Chojnacki, T., Danikiewicz, W., Swiezewska, E., 2003. Divergent pattern of polyisoprenoid alcohols in the tissues of *Coluria geoides*: a new electrospray ionization MS approach. Lipids 38, 981–990.

Skowronek, M., Jemioła-Rzemińska, M., Kruk, J., Strzałka, K., 1996. Influence of the redox state of ubiquinones and plastoquinones on the order of lipid bilayers studied by fluorescence anisotropy of diphenylhexatriene and trimethylammonium diphenylhexatriene. Biochim. Biophys. Acta 1280, 115–119.

Slotte, J.P., 2009. Lipid interactions, domain formation, and lateral structure of membranes. Biochim. Biophys. Acta 1788, 1–9.

Stone, K.J., Wellburn, A.R., Hemming, F.W., Pennock, J.F., 1967. The characterization of ficaprenol-10-11 and 12 from the leaves of *Ficus elastica* (decorative ruber plant). Biochem. J. 102, 325–330.

Swiezewska, E., Dallner, G., Andersson, B., Ernster, L., 1993. Biosynthesis of ubiquinone and plastoquinone in the endoplasmic reticulum–Golgi membranes of spinach leaves. J. Biol. Chem. 268, 1494–1499.

Swiezewska, E., Danikiewicz, W., 2005. Polyisoprenoids: structure, biosynthesis and function. Prog. Lipid Res. 44, 235–258.

Valtersson, C., Van Duijn, G., Verkleij, A.J., Chojnacki, T., De Kruijff, B., Dallner, G., 1985. The influence of dolichol, dolichol esters, and dolichyl phosphate on phospholipid polymorphism and fluidity in model membranes,. J. Biol. Chem. 260, 2742–2751.

Vigo, C., Grossman, S.H., Drost-Hannsen, W., 1984. Interaction of dolichol and dolichyl phosphate with phospholipid bilayers. Biochim. Biophys. Acta 774, 221–226.

Wang, X., Mansourian, A.R., Quinn, P.J., 2008. The effect of dolichol on the structure and phase behaviour of phospholipid model membranes. Mol. Membr. Biol. 25, 547–556.

Weinstein, J.N., Klausner, R.D., Innerarity, T., Ralston, E., Blumenthal, R., 1981. Phase transition release, a new approach to the interaction of proteins with lipid vesicles. Application to lipoproteins. Biochim. Biophys. Acta 647, 270–284.

Zhou, G.P., Troy II, F.A., 2003. Characterization by NMR and molecular modeling of the binding of polyisoprenols and polyisoprenyl recognition sequence peptides: 3D structure of the complexes reveals sites of specific interactions. Glycobiology 13, 51–71.

Zhou, G.P., Troy, F.A., 2005. NMR study of the preferred membrane orientation of polyisoprenols (dolichol) and the impact of their complex with polyisoprenyl recognition sequence peptides on membrane structure. Glycobiology 15, 347–359.